

## **REMARKS**

### **I. Status of the Claims**

Claims 1, 4, 6, 10-27, 30-48 and 71 are pending and under consideration, claims 2, 3, 5, 7-9, 28, 29 and 49-70 having been previously canceled. With this Amendment, claims 1 and 27 are being amended, and claim 4 is canceled, without prejudice against its reintroduction into this or one or more timely filed continuation, divisional or continuation-in-part applications. Thus, after entry of this Amendment, claims 1, 6, 10-27, 30-48 and 71 remain pending and under consideration. The amendments of the claims and the various rejections raised in the Office Action are discussed in more detail, below.

### **II. Amendments**

Claims 1 and 27 are amended to incorporate the limitation of claim 4.

No new matter is added by way of these amendments.

### **III. Rejection under 35 U.S.C. §112, second paragraph**

Claim 4 is rejected under 35 U.S.C. §112, second paragraph as allegedly indefinite.

The rejection of this claim is obviated by its cancelation. Applicants respectfully request withdrawal of the rejection under 35 U.S.C. §112, second paragraph.

### **IV. Rejections under 35 U.S.C. §103(a)**

Claims 1, 4, 6, 10-27, 30-48 and 71 were rejected under 35 U.S.C. §103(a) as allegedly obvious over Schmukler (of record) in view of Gutierrez-Armenta (of record) and Dev (of record).

Claims 1, 4, 6, 10-27, 30-48 and 71 were rejected under 35 U.S.C. §103(a) as allegedly obvious over Dev (of record) in view of Porter (US 2,932,128).

These rejections are respectfully traversed.

#### **A. The Present Claims**

As amended, the present claims refer to a method for improving the efficiency of transfer of a nucleic acid into a plant cell having an intact cell wall and contained in a seed, comprising the steps of a) placing a seed containing the cell and the nucleic acid in a container and depressurizing the container wherein the pressure in the container is reduced by about 0.096 MPa from the atmospheric pressure; b) subsequently placing the seed containing the cell and the nucleic acid under conditions to induce electroporation; and c) transferring the nucleic acid into the plant cell using electroporation (Claim 1); and a method for improving the efficiency of introducing a nucleic

acid into a cell of a plant, wherein the cell has an intact cell wall, comprising the steps of a) placing a seed containing the cell and the nucleic acid in a container and depressurizing the container wherein the pressure in the container is reduced by about 0.096 MPa from the atmospheric pressure; b) subsequently placing the seed containing the cell and the nucleic acid under conditions to induce electroporation and introducing the nucleic acid into the cell using electroporation; and c) differentiating, growing, and/or multiplying the cell (Claim 27).

#### B. The Cited Art

SCHMUKLER discloses an apparatus and method for electroporation and electrofusion of cells, in particular myeloma and lymphoma cells, which are types of animal cells, as well as isolated nuclei (see Col . 3 of Schmukler).

GUTIERREZ-ARMENTA discloses the use of retinoblastoma protein to control growth of plant cells and/or plant viruses. Several methods of administering nucleotides to cells are disclosed, including electroporation of plant seed cells with DNA.

DEV disclose a method for producing genetically modified plants via electroporation in the absence of cell-wall degrading enzymes.

PORTER discloses inoculation of seeds with bacteria or particulate matter.

#### C. Analysis

C1. Rejection of claims 1, 4, 6, 10-27, 30-48 and 71 as allegedly obvious over Schmukler, in view of Gutierrez-Armenta and Dev

The Examiner appears to be overlooking several elements required by Applicants' present claims: the claims require that the plant cell has an intact cell wall, is contained within a seed which is placed in a container with the nucleic acid, and depressurization of the container.

First, Schmukler teaches a method of electroporation of **cells** trapped in a film having pores with diameters smaller than the diameters of the cells, before an electric field is applied to cause electroporation. Schmukler is generally directed to electroporation and electrofusion of animal cells, as well as isolated nuclei, which lack the cellulose cell walls of plant cells. The Examiner points to column 3, lines 44-47 as teaching exposure to depressurization across the film containing the pores; however, (see column 3, lines 27-34) the method of Schmukler requires that the cell is deformed such that a portion of the cell extends substantially into the pore of the membrane. Such a deformation would not occur in plant cells having cellulose cell walls, much less in a seed which contains the cell.

Second, Schmukler teaches establishment of a pressure gradient across the cell membrane to trap cell on a film having pores, to force the cells to protrude through the pores, and to force material in or out through the cell membrane. This pressure gradient described in Schmukler differs from the claimed seed containing the cell in a container depressurization of the ambient environment in the container containing the nucleic acid and a plant **seed** containing a cell with an intact cell wall of the present claims.

Finally, Schmukler's system does not require the container as presently claimed, in which the seed is placed for the depressurization, and thus Schmukler would not be able to achieve the presently claimed level of depressurization.

Neither Gutierrez-Armenta nor Dev supplies the missing teachings. Specifically, neither Gutierrez-Armenta nor Dev teaches electroporation of DNA into a plant cell having an intact cell wall and contained in a seed which is placed in a container with the nucleic acid, depressurizing the container wherein the pressure in the container is reduced by about 0.096 MPa from the atmospheric pressure. These references fail to teach, suggest or enable the concept of transformation of a nucleic acid through an intact plant cell wall.

C2. Rejection of claims 1, 4, 6, 8-27, 29-48 and 71 as allegedly obvious over Dev in view of Porter

The deficiencies of the Dev reference are described above.

The Porter reference fails to qualify as analogous prior art. In order to qualify, "the reference must be either in the field of applicant's endeavor or, if not, then be reasonably pertinent to the particular problem with which the inventor was concerned." (See *In re Oetiker*, 977 F.2d 1443 (Fed. Cir. 1992)). The Porter reference is from a field quite distinct from that of Applicants' subject matter, as it is directed to inoculation of seeds with bacteria or particulate matter, wherein the bacteria or particulate matter is incorporated beneath the surface of the seed, and fails to teach or even disclose electroporation, much less the introduction of DNA into a plant cell having an intact cell wall. Porter further fails to teach placing a seed containing the cell and a nucleic acid in a container and depressurizing the container. Again, it appears that the Examiner is arriving at a finding of obviousness based only on hindsight reconstruction, cobbling together elements from disparate references that the skilled artisan would not reasonably combine.

Thus, in considering the disclosure of references as a whole, Applicants submit that the skilled artisan would not have, without benefit of hindsight, combined the electroporation method described in Dev with the method described in Porter.

Because the cited references, when considered either singly or in combination, fail to teach, enable or suggest the presently claimed method, and could not lead one skilled in the art to expect to achieve the presently claimed method, the standard for obviousness has not been met. Accordingly, Applicants respectfully request withdrawal of the rejection under 35 U.S.C. §103.

**CONCLUSION**

In view of the foregoing, claims 1, 6, 10-27, 30-48 and 71 are believed to satisfy all of the criteria for patentability and are in condition for Allowance. An early indication of the same is therefore kindly requested.

No fees are believed to be due in connection with this Amendment. However, the Commissioner is authorized to charge any additional fees that may be required, or credit any overpayment, to King & Spalding LLP Deposit Account No. 50-4616.

If, in the opinion of the Examiner, a telephone conference would expedite the prosecution of the subject application, the Examiner is encouraged to call the undersigned at (650) 590-1932.

Respectfully submitted,  
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